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ION EFFECTS IN X-RAY DEPOLARIZATION OF MUSCLE MEMBRANE

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### Introduction:

Irradiation of frog sartorius muscles with 100 Kr of X-rays results in a decrease in the resting membrane potential of  $20 \pm 0.5$  mV when the fibers are bathed in standard Ringer's solution (112 mM NaCl, 2.5 mM KCl, 1.8 mM  $\text{CaCl}_2$ , 2.5 mM  $\text{Na}_2\text{HPO}_4$ , 0.5 mM  $\text{NaH}_2\text{PO}_4$ ). This depolarization occurs during the irradiation period. After irradiation, the potential is constant for at least 6 hours (1). Irradiation thus alters the properties of the muscle cell membrane, and therefore provides an additional method for studying these properties. In Part I, the effects on this irradiation depolarization of altering the sodium and potassium ion concentrations in the bathing fluid are presented. The results show that the depolarization is primarily due to an increase in sodium permeability of the muscle. Since calcium ions have been widely implicated in the control of cell membrane permeability, especially permeability to sodium ions (2), the rate of loss of radioactive calcium from muscles has been studied in Part II, as a function of both irradiation and stimulation.

### PART I

#### Methods:

Sartorius muscles ranging from 90 to 100 mg. fresh weight were dissected carefully so as to leave the lower side as free as possible from connective tissue. One muscle from each frog was used for irradiation, one as a control. Nine frogs were used for each solution. The muscles were mounted in a special holder at 120% of their rest length by a micrometer system. Muscles were equilibrated for 60 minutes in 500 ml. of the experimental solution prior to microelectrode

penetration. The solution was not "aerated" with the usual  $O_2$ - $CO_2$  gas mixture in order to minimize mechanical damage to the surface of the muscle cells. All experiments were carried out at  $25 \pm 0.5^\circ C$  and pH 7.3.

Membrane potentials were measured with 3 M KCl-filled glass micropipette electrodes having resistances between 10 and 12 megohms. The electrical measurements were made with the conventional equipment for this work. Only potentials that developed instantaneously with penetration of the muscle cell were recorded and used in the data. Each plotted point in the figures represents an average of potential measurements on 130 cells. The standard error of the mean is less than 0.5 mV. Muscles were irradiated while mounted in the chamber at 6,000 r/min. to a total dose of 100 Kr, using a G. E. Maxitron X-ray machine at 300 KVP, 0.25 mm Al filter.

#### Results:

Figure 1 shows the results of one set of experiments in which standard (112 mM) and one-half standard (56 mM NaCl, 56 mM choline chloride) sodium concentration were used, each containing no potassium. The resting membrane potential in control fibers is independent of sodium concentration and time. In irradiated fibers, the potential after irradiation depends on the sodium concentration, and decreases with time after irradiation at a rate of 3.4-3.8 mV per hour. The ratio of control to irradiated potentials immediately after irradiation is 1.56 in 112 mM sodium, 1.28 in 56 mM sodium.

Figures 2 and 3 show the results of sets of experiments using the same two sodium concentrations as above, with potassium concentrations of 1, 2.5, 10, 20, 50, and 80 mM. With potassium present

in the bathing solution, the potential no longer decreases with time after irradiation,  $dv/dt = 0$ . Furthermore, the ratio of the control to irradiated potentials is 1.22-1.25 in 112 mM sodium solutions, and 1.12-1.13 in 56 mM solutions, at all potassium concentrations. The membrane potential in controls is independent of  $[Na]_o$ .

Figure 4 summarizes the results obtained in a series of similar experiments in which the potassium concentration was varied from 1 to 80 mM. The points labelled  $\circ, \bullet$  represent potentials observed when the sodium concentration was reduced, for potassium concentrations above 30 mM, in order to maintain the osmolar concentration of the bathing solutions constant. The points labelled  $\odot, \ominus$  represent potentials observed when the sodium concentration was maintained at 112 mM so that the total osmolar concentration increased as the potassium concentration increased. The converging straight lines obtained for control measurements (slope for 10-fold K: 50.5 mV), measurements after irradiation in standard sodium at constant osmolarity (slope for 10-fold K: 40.2 mV), and at one-half standard sodium at constant osmolarity (slope for 10-fold K: 45.5 mV; the osmolarity was adjusted with choline chloride) show very clearly that the ratio of control to irradiated potentials is constant, and close to 1.25 in standard sodium, 1.12 in one-half sodium, regardless of potassium concentration above 1.0 mM. That is, the fractional depolarization produced by irradiation is independent of potassium concentration above 1.0 mM, but strongly dependent on sodium concentration.

#### Discussion:

The clear-cut dependence on external sodium concentration of the irradiation induced depolarization and its independence of external

potassium concentration suggest very strongly that the depolarization is the result of an increased sodium permeability. Furthermore, we conclude that irradiation produces no appreciable increase in potassium permeability. We have previously reported that 100 Kr X-irradiation of frog sartorius does increase the potassium efflux by 50%, but this is just what we should expect as a result of the change in membrane potential. If the potassium efflux follows the relationship

$$J_k = \frac{P_k \cdot F}{R \cdot T} \cdot [K]_i \cdot V_m / (e^{V_m/RT} - 1)$$

and if only the membrane potential is altered by irradiation, then the calculated ratio of irradiated to control potassium effluxes in 2.5 mM potassium Ringer's is 1.6. The measured value (1) was 1.5. Since any increase in potassium permeability would lead to a calculated ratio even higher than 1.6, we conclude that there is no significant increase in  $P_K$  with irradiation. The maintained increase of 12% in membrane conductance after irradiation reported in ref 1. indicates that the permeability to some ion must have increased, and it seems most likely that ion is sodium.

It is also noteworthy that the stability of the membrane potential after irradiation in 1.0 mM and higher potassium concentrations implies that the sodium extrusion mechanisms can keep up with the increased sodium influx after irradiation, but cannot exceed it. In fact, the constancy of the two different potentials at the two different sodium concentrations suggests that the activity of the sodium extrusion system is very precisely matched to the rate of sodium influx, since the latter must be less in the lower sodium concentration solutions. Similarly, the continued decrease in potential after irradiation in zero potassium solutions suggests that



without any external potassium the sodium extrusion systems can no longer keep up with sodium influx after irradiation, although they can in control fibers (Figure 1). It thus appears that irradiation causes damage to the muscle cell as a result of which increased sodium influx occurs. The ability of the membrane to extrude sodium is retained, but it has become potassium dependent.

## PART II

### Methods:

Frog sartorius muscles between 25 and 30 mg. were carefully dissected out in pairs (one control, one irradiated), mounted in a special isometric chamber at 120% of rest length, and soaked in standard Ringer's solution for 15 minutes. They were then "loaded" with  $\text{Ca}^{45}$  by soaking for two hours in a solution of the same composition containing 1.78  $\mu\text{curies/ml. of Ca}^{45}$ . After a three minute wash in non-radioactive Ringer's solution to remove superficial radioactive solution, all muscles had initial activities of  $11 \times 10^{-8}$  Moles  $\text{Ca}^{45}$  per gm. wet weight (S.E.M. :  $1.5 \times 10^{-8}$  Moles). Ten pairs of muscles were used for each experiment. The two hour loading period was chosen for two reasons. First, trial experiments showed that no increase in level of radioactivity occurred with longer soaking periods. Second, the muscle nerves were severed during dissection as close to the muscle as possible so that degeneration of the motor end-plates occurred in about 40 minutes. After this time, no miniature end-plate potentials and no spontaneous twitches were observed.

Calcium loss was measured by soaking each muscle in 5 ml. of appropriate Ringer's solutions, and changing to fresh solution every

20 minutes. An appropriate aliquot of each 5 ml. solution was evaporated to dryness in a planchet and counted in a gas flow counter. Irradiation was carried out as described earlier (1, 2), during the period from 43 to 63 minutes after removal from the  $\text{Ca}^{45}$  loading solution. When required, stimulation was with supramaximal 10 msec. rectangular pulses at one per second for a ten minute period. Mechanical responses were recorded through a Grass FT03 transducer connected to one end of the muscle. All measurements were carried out at 25°C and in Ringer's solutions of several different compositions, maintained at pH 7.3, as indicated in Table 1. For each set of experiments, wet weights were measured at the beginning and end of the experiments. At the end of each experiment, dry weight, ash weight and total  $\text{Ca}^{45}$  content were also determined for each muscle (4).

#### Results:

The results of these measurements are shown in Figures 5, 6, and 7. Neither irradiation nor stimulation, nor the combination of both, results in any significant change in the rate at which  $\text{Ca}^{45}$  is lost from muscle fibers under these conditions. Substitution of bicarbonate for phosphate, or omission of either buffer also resulted in no significant effect. Comparison of curves in Figure 5 shows that the rate of loss of  $\text{Ca}^{45}$  is higher if there is no calcium in the external solution, but this increased rate of loss is not affected by irradiation or stimulation.

#### Discussion:

The result that  $\text{Ca}^{45}$  is lost more rapidly into calcium-free solutions indicates that the  $\text{Ca}^{45}$  loss measured in these experiments is not primarily dependent on an exchange reaction, since in that case

the rate should be lower in the calcium-free solution. The absence of any radiation effect, in view of the depolarization and increased sodium influx known to be occurring after irradiation in these fibers (Part I), makes it clear that displacement of calcium from some sort of bound condition to a state in which it is free to diffuse out of the membrane cannot be the mechanism for the increase in sodium permeability occurring with irradiation. The stimulation experiments lead to a similar conclusion with respect to excitation. These results do not necessarily imply that depolarization is independent of calcium in the membrane. Calcium ions could be dislocated from binding at one type of site to binding at another without being released from the membrane itself. Furthermore, it is not clear to what extent the depolarization produced by irradiation is analogous to that produced by excitatory mechanisms. These results merely show that neither type of depolarization results in a change in the rate of release of calcium. Further studies are in progress to determine whether the depolarization induced by raising the external potassium concentration produces any change in rates of  $\text{Ca}^{45}$  release. The mechanisms by which radiation increases sodium permeability remain unknown.

Summary:

Irradiation of frog sartorius muscles with 100 Kr of X-rays results in a relative decrease in resting membrane potential which is dependent on external sodium concentration, but not on potassium concentration provided that the latter exceeds 1.0 mM. In zero potassium solutions, the potential decreases more, and continues to fall after irradiation. Neither irradiation nor stimulation alters the rate at which  $\text{Ca}^{45}$  is lost from previously loaded muscle fibers. These results

are interpreted to mean that irradiation damage to the muscle cell membrane results in increased permeability to sodium, but not via the release of calcium ions. The sodium extrusion mechanisms are altered by irradiation so as to require an external potassium concentration above 1.0 mM to match the increase of Na influx. Permeability to potassium does not appear to be altered by irradiation.

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Figure 1. - The membrane potential measured at 2 external concentrations of Na, OmmK is plotted against the time in contact with the Ringer solution.

○control; ● irradiated (112 mMNa)  
●control; ● irradiated ( 56 mMNa)  
I.P.: Irradiation Period  
E.P.: Equilibration Period

Figure 2. - The membrane potential measured at several external concentrations of K in function of time. The fractional depolarization, dependent on Na concentration, is demonstrated in the experiments at 2 external Na concentrations, 10 mM.

○control; ● irradiated (112 mMNa)  
●control; ● irradiated ( 56 mMNa)

Figure 3. - The membrane potential at 20 and 50 mM external concentrations of K.

○control; ● irradiated (112 mMNa)  
●control; ● irradiated ( 56 mMNa)

Figure 4. - The membrane potential is plotted against the logarithm of the external concentration of K.

A. ○control; ● irradiated. (KCl is substituted for NaCl, in Ringer fluid).

●control, half Na; ● irradiated, half Na. (These symbols represent half Na from the external concentrations of Na used above; the osmolar concentration of the solutions was maintained by adding choline chloride).

B. ●control; ● irradiated (Na concentration was maintained at 112 mMNa. The total osmolar concentration increased as the K concentration increased).

Figure 5. - Ca 45 released in Sartorius muscles soaked respectively in Ringer's solutions A and B.

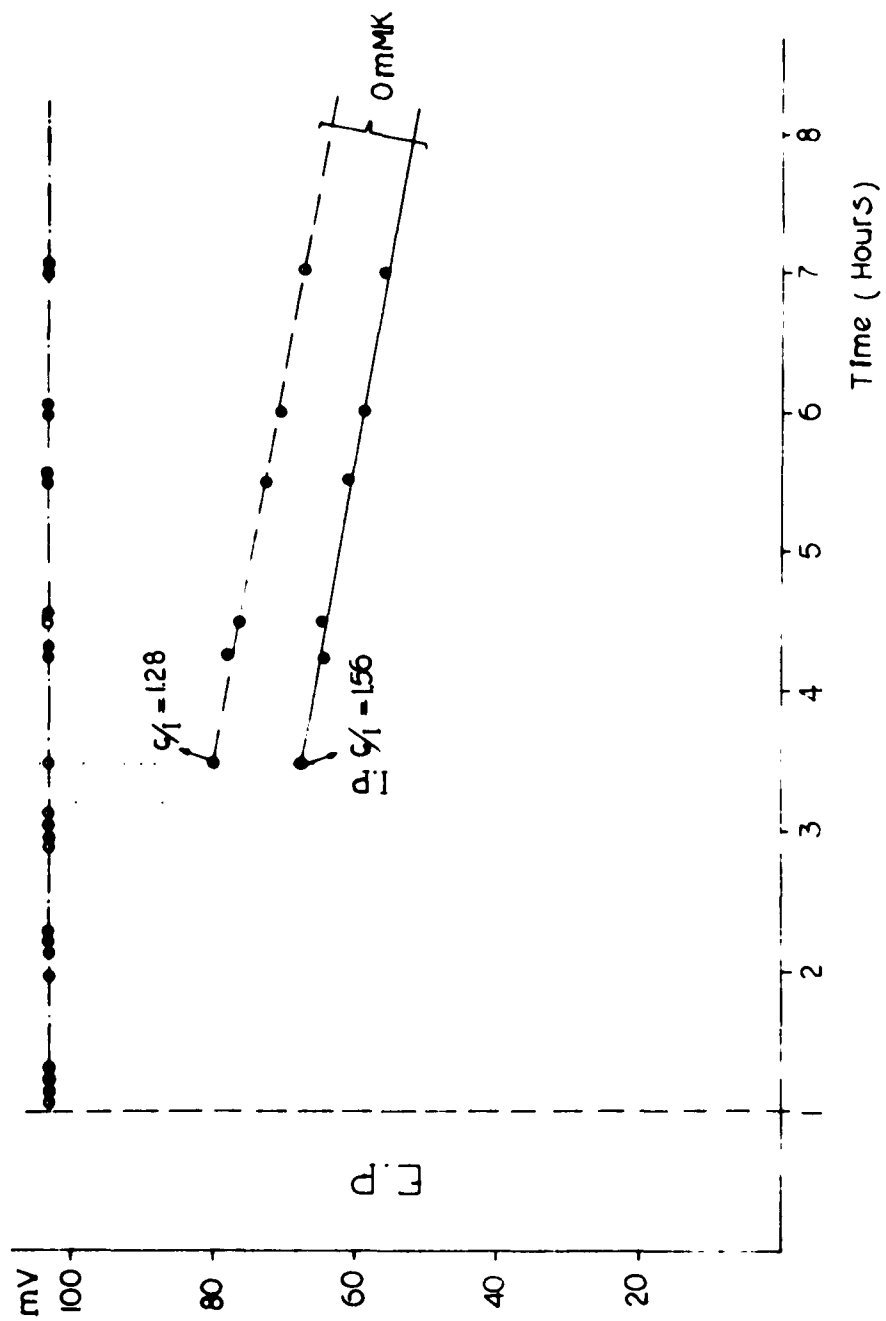
Figure 6. - Ca 45 released in Sartorius muscles soaked respectively in Ringer's solutions C and D.

Figure 7. - Ca 45 released in Sartorius muscles soaked in Ringer D, as shown in Figure 6, but with a period of electrical stimulation following that of irradiation.

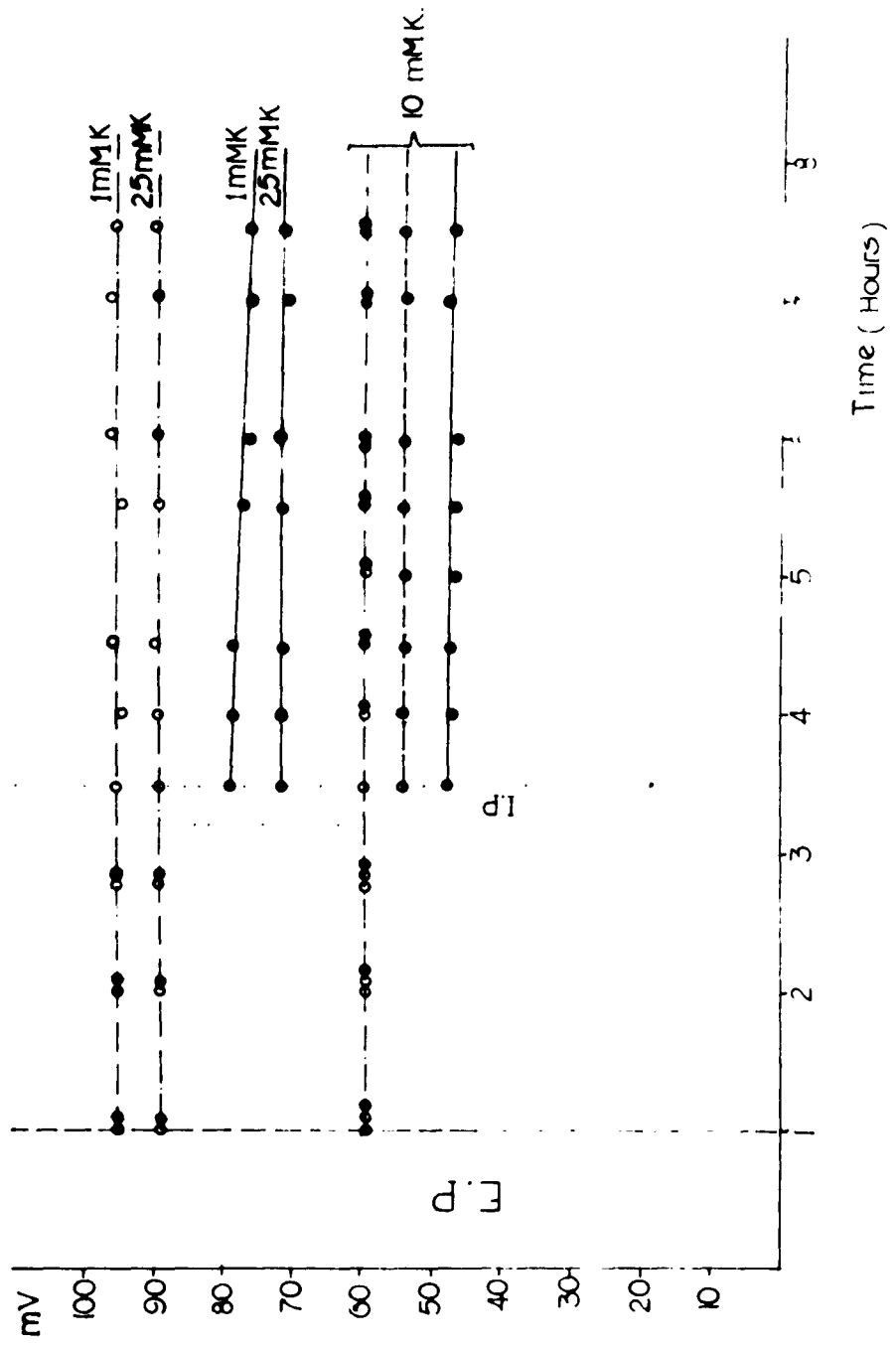
× stimulated; • control (This set of muscles was not irradiated).

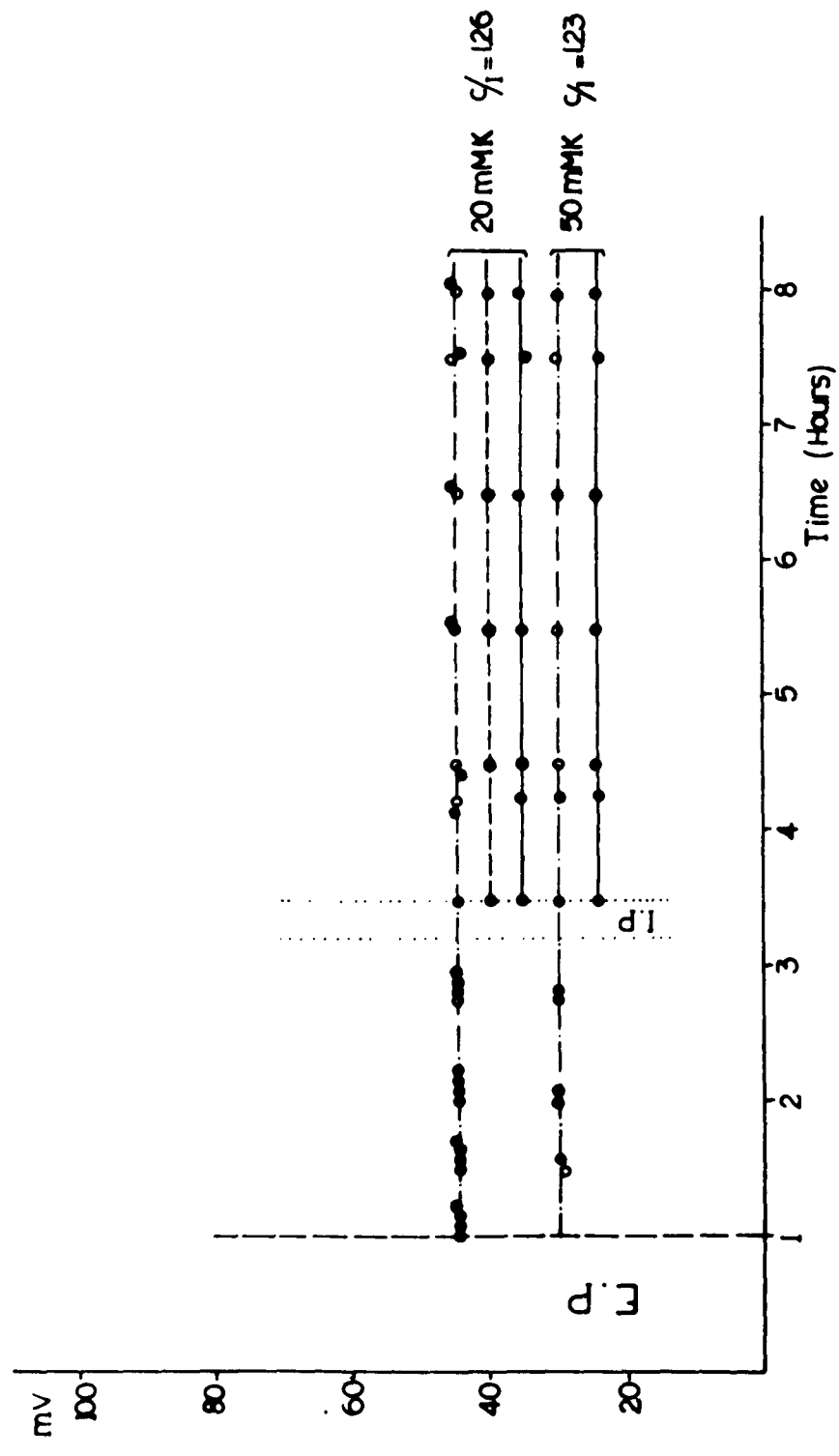
▴ irradiated and stimulated

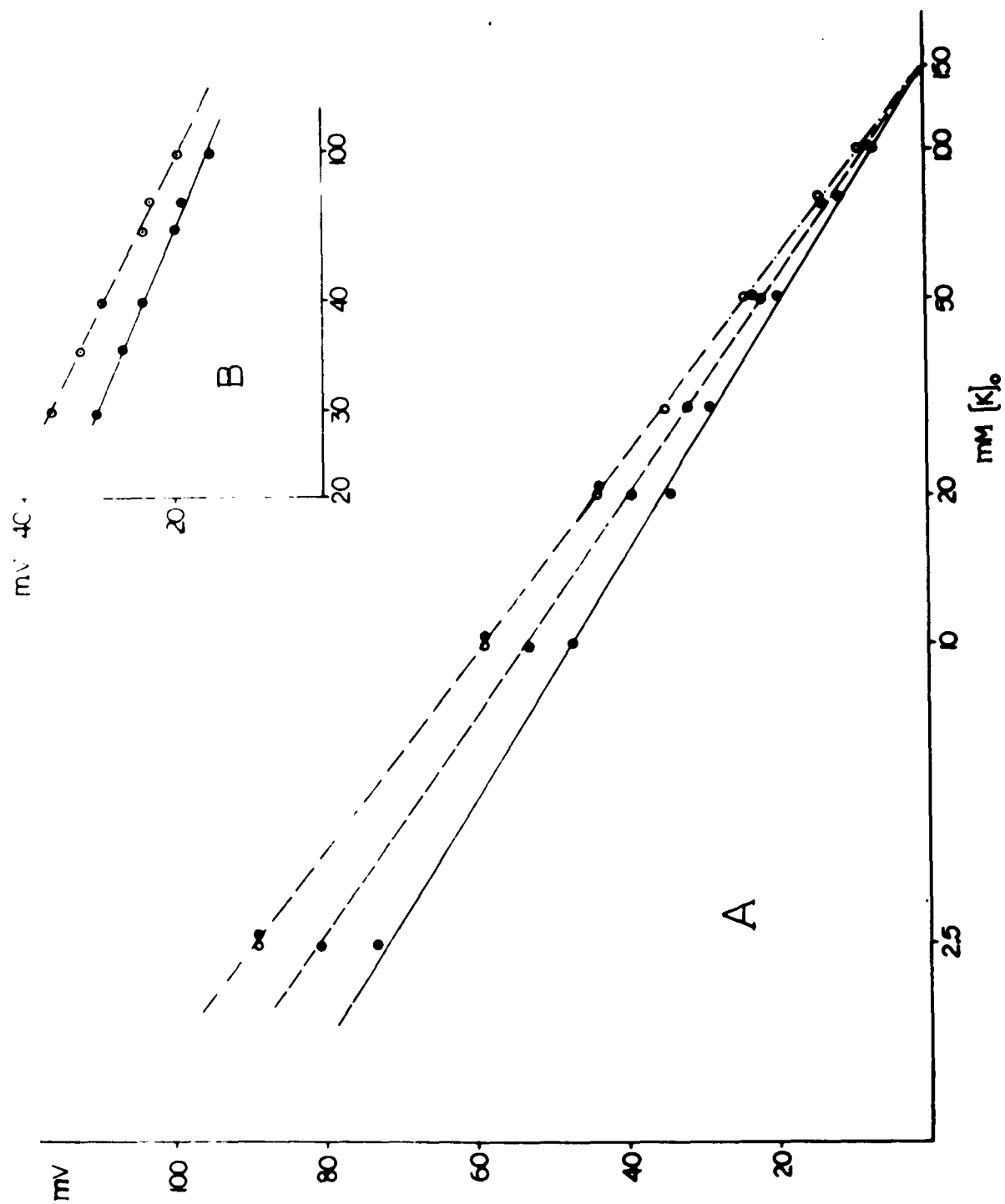
○ control; non-irradiated, non-stimulated











| Composition<br>of solutions<br>(mM) | A     | B     | C     | D     |
|-------------------------------------|-------|-------|-------|-------|
| NaCl                                | 112.0 | 112.0 | 112.0 | 112.0 |
| KCl                                 | 2.5   | 2.5   | 2.5   | 2.5   |
| CaCl <sub>2</sub>                   | 1.89  |       | 1.89  | 1.07  |
| Na <sub>2</sub> HPO <sub>4</sub>    |       |       | 2.50  |       |
| NaH <sub>2</sub> PO <sub>4</sub>    |       |       | 0.50  |       |
| NaHCO <sub>3</sub>                  |       |       |       | 2.38  |
| K <sub>2</sub> HPO <sub>4</sub>     |       |       |       | 0.08  |

